



## Addition of Oil Palm Mistletoe Leaf Extract (*Ficus heteropleura* Blume) in Membrane Production and Antibacterial Testing of *Escherichia coli*

Yola suryani<sup>1</sup>, Fitra Perdana<sup>\*1,2</sup>, Jufrizal Syahri<sup>1</sup>

<sup>1</sup>Department of Chemistry, Faculty of Mathematics, Natural Sciences, and Health, Universitas Muhammadiyah Riau  
Jl. Tuanku Tambusai Pekanbaru, 28294, Riau, Indonesia

<sup>2</sup>Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Riau  
Jl. HR Soebrantas Pekanbaru, 28293, Riau, Indonesia

Received 22 November 2023 | Accepted 11 November 2024 | Published 30 November 2024  
DOI: <https://doi.org/10.37859/jp.v15i1.6262>

### Keywords:

Palm mistletoe leaf extract;  
Phytochemical test  
Anti-biofouling;  
Membrane;  
*Escherichia coli*

**Abstract.** Palm mistletoe leaves (*Ficus heteropleura* Blume) are a plant that is effective as an antibacterial medicine. Palm mistletoe leaves contain antibacterial compounds such as flavonoids, tannins, saponins and phenolics. One alternative to reduce fouling is adding an anti-biofouling agent to the membrane. This research aims to determine the phytochemical test on oil palm mistletoe leaf extract, to see the antibacterial inhibition zone of *Escherichia coli* and determine the characteristics of thickness and biodegradation, as well as the anti-biofouling properties of natural ingredients, namely oil palm mistletoe leaves. The addition of palm mistletoe leaf extract had concentrations of (4:0), (3.96:0.04), and (3.92:0.08) grams. For the antibacterial results obtained by adding a membrane to the additive, namely palm mistletoe leaves, namely variation 1, has an average diameter of 4.73 mm, 4.3 mm and 4.33 mm. The positive control concentration had an average diameter of 38.3 mm. In variation 2 the measurement results do not show the existence of an inhibition zone. The measurement results of the positive control had an average diameter of 38.367 mm. For membrane thickness testing, namely 0.03 mm, 0.06 mm, and 0.06 mm, and the highest biodegradation test was found in the variation of adding 0.08 gram extract, namely 56% and the lowest biodegradation value was in the variation of adding 0.04 gram extract namely 35%.

\*Corresponding author.

E-mail address: [fitraperdana@umri.ac.id](mailto:fitraperdana@umri.ac.id)

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## 1. Introduction

Indonesia has a rich biodiversity that has great potential for use in the field of health. Plants in Indonesia have long been used as traditional medicine. WHO also recommends maintaining health and treating diseases using traditional medicine. Consuming traditional medicine is considered safer than using chemical drugs (Kumalasari & Andiarna, 2020). Plants usually contain secondary metabolites, such as alkaloids, flavonoids, triterpenoids/steroids, saponins, tannins, and phenolics (Najoan et al., 2016). Membrane technology in the separation process has developed rapidly. Membranes can specifically limit the transport of various chemical species and separate two phases (feed and permeate). Membrane separation is a technology that has been widely developed as an alternative to conventional separation processes. The technology has advantages compared to conventional techniques, such as low energy consumption, the ability to be combined with other separation processes, no need for additives, and suitability for compounds that are not heat-resistant. With these advantages, membrane technology has been widely applied in various fields, including industry, chemistry and pharmaceuticals, waste treatment and environmental technology, and oil processing, specifically in the processing of vegetable oil (Husni et al., 2018). Based on structure, membranes are classified into two types: symmetric and asymmetric membranes. There is a dialysis process, which uses pressure as the driving force, usually employing asymmetric membranes. Various methods have been used in the membrane fabrication process, such as sintering, stretching, tracketching, template leaching, coating, and phase inversion. Phase inversion has become a popular method in developing asymmetric structured membranes. Phase inversion is the process of changing the form of a polymer from a single liquid phase to a two-liquid phase (liquid-liquid demixing). The mechanism of membrane formation is influenced by the concentration of the polymer, the type of solvent-nonsolvent, and the addition of additives (Lusiana & Prasetya, 2020).

Cellulose has advantages such as abundant raw materials, biodegradability, good mechanical strength, high thermal stability, high electrochemical stability, and the ability to be easily formed into sheets. The cellulose derivative that is widely used in the industry is cellulose acetate (Ginting et al., 2023). Acetate cellulose is a cellulose derivative compound obtained through esterification using acetic anhydride as a reagent. Acetate cellulose is one type of polymer that is widely used in the industry. Cellulose acetate is generally classified into two types: triacetate cellulose (polymer cellulose acetate) and diacetate cellulose (secondary cellulose acetate). Primary cellulose acetate is made through the esterification (acetylation) reaction of cellulose with acetic anhydride, while secondary cellulose acetate is made by hydrolyzing primary cellulose acetate (Syamsu & Kuryani, 2014). The obstacles that occur during the membrane filtration process can be caused by the presence of substances in the solution that block the pores (fouling). One type of fouling that often occurs is biofouling. The complex layer that adheres to the membrane surface can consist of microorganisms that form a biofilm (microfouling) (Alvianto et al., 2022). Coating the membrane with natural materials such as oil palm mistletoe leaves can reduce fouling that can damage the membrane. Palm oil tree mistletoe leaves are known to have antibacterial properties due to their phenolic compound content.

This research uses the maceration method for the extraction of oil palm mistletoe leaves. Maceration is one of the most commonly used extraction methods, involving placing plant powder and an appropriate solvent into a tightly sealed container at room temperature. However, there are also major drawbacks to this method, such as it being time-consuming, requiring a significant amount of solvent, and the likelihood that some compounds may be lost. Additionally, some compounds may be difficult to extract at room temperature, but the maceration method can also avoid the risk of degradation of thermolabile compounds in the plant (Badaring et al., 2020). The extract of oil palm mistletoe leaves added to the production of cellulose acetate membranes can reduce the antibacterial activity against *Escherichia coli*. So far, research on the addition of extracts in membrane production has focused on the characteristics of membrane performance and antifouling properties applied to

bacteria. This research aims to determine the phytochemical tests on the extract of palm oil mistletoe leaves, to observe the antibacterial inhibition zone against *Escherichia coli*, and to understand the characterization of thickness and biodegradation, as well as the anti-biofouling properties using natural materials, namely palm oil mistletoe leaves.

## **2. The Methods**

### **2.1. Tools and materials**

In this study, the tools used are dark immersion bottles for leaf extract, distillation, rotary evaporator vacuum, beaker glass, funnel, petri dish, test tube, test tube rack, spatula, stirring rod, analytical balance, hotplate stirrer, autoclave, incubator, glass plate, casting knife, screw micrometer, cotton, rubber, measuring cup, oven, plastic wrap and Whatman filter paper, and Laminar Air Flow. The materials used are palm oil mistletoe leaves, cellulose acetate, 96% technical ethanol solvent, 1 M HCl, Mayer's reagent, 70% ethanol, ether, concentrated H<sub>2</sub>SO<sub>4</sub>, concentrated HCl, magnesium powder, aquadest, 1% FeCl<sub>3</sub>, 5% FeCl<sub>3</sub>, N,N-Dimethylacetamide (DMAc), NA, aquades, *Escherichia coli* bacteria, and aluminum foil.

### **2.2. Preparation extract**

The samples of oil palm mistletoe leaves are washed and cleaned to remove dust, then dried at room temperature for 1-2 weeks. After drying, the leaves are ground with a blender until they form a powder. As much as 250 g of palm mistletoe leaf powder was extracted by maceration using 96% ethanol solvent until all samples were submerged and for 3 days with occasional stirring. After 3 days, the macerated sample is filtered. The filtrate obtained from the maceration process is concentrated using a vacuum rotary evaporator (Khasanah et al., 2020).

### **2.3. Phytochemical test**

#### *2.3.1. Alkaloid test*

0.05 g of the sample is placed into a test tube, dissolved with 10 ml of 1 M HCl solution. After that, it is filtered. 4 ml of the filtrate was placed into a test tube and 1 ml of Meyer's reagent was added. The formation of a yellowish-white precipitate indicates a positive test result (Khasanah et al., 2020).

#### *2.3.2. Saponin test*

0.05 g of the sample is placed into a test tube, added with 70% ethanol. Then warm water is added. For 30 minutes, the tube was shaken. The foam is observed and its diameter is measured, then it is left for 10 minutes. If there is no foam, concentrated HCl is added. If the foam remains, it indicates a positive result.

#### *2.3.3. Tannin test*

0.05 g of the sample is placed into a test tube and added with 10 ml of boiling aquades, then the sample is filtered. There is a greenish-brown or bluish-black color when 2-3 drops of 1% FeCl<sub>3</sub> solution are added to the filtrate. This test is considered positive.

#### *2.3.4. Phenolic test*

0.05 g of the extracted sample was added to 20 ml of 70% ethanol. 1 ml of the extract was taken and 2 drops of 5% FeCl<sub>3</sub> solution were added. The green or blue color indicates the presence of phenolic compounds in the extract.

#### *2.3.5. Flavonoid test*

0.05 g of the sample was extracted with 5 ml of 70% ethanol, heated in a test tube for approximately 5 minutes. Then, 3 drops of concentrated HCl and 0.2 g of Mg powder were added. The dark red, or magenta, color indicates the presence of flavonol or flavanon compounds, the red precipitate indicates flavon compounds, and the green precipitate indicates glycoside or glycon compounds.

### 2.3.6. Steroid/triterpenoid test

0.05 g of extract is placed into a test tube, added with 70% ethanol. Then, 5 drops of ether were added until two layers of aqueous and ethanol solution were formed. The upper layer of ethanol was separated and evaporated on a watch glass before adding H<sub>2</sub>SO<sub>4</sub>. The dark green precipitate indicates a positive result.

## 2.4. Membrane fabrication

The production of membranes as antifouling agents is carried out by mixing cellulose acetate, DMAc, and an additive in the form of palm oil mistletoe leaf extract (Alvianto et al., 2022).

**Table 1.** Composition of the samples

| Variation    | Extract (grams) | CA (grams) | N,N – Dimethylacetamide (ml) |
|--------------|-----------------|------------|------------------------------|
| CA           | -               | 4          | 20                           |
| CA:Extract-1 | 0.04            | 3.96       | 20                           |
| CA:Extract-2 | 0.08            | 3.92       | 20                           |

The production of membranes as antifouling agents is carried out by mixing cellulose acetate, DMAc, and an additive in the form of palm oil mistletoe leaf extract, as shown in Table 1. All membrane compositions were homogenized using a hot plate magnetic stirrer at 500 rpm for 2 hours in a glass beaker. The mixture of the three homogenized materials was left to stand for 24 hours to eliminate bubbles in the solution. The solution that has been left to stand for 24 hours. Then, a 10 ml liquid sample was taken for FTIR testing, antibacterial testing, and the remaining part was poured onto a glass plate that had been cleaned with ethanol solvent and smoothed using a casting knife. The solution that has been cast is dipped in a coagulation bath containing ethanol for 10 minutes until a thin membrane sheet is formed. The function of soaking in ethanol as a non-solvent material is to diffuse into the underside of the membrane, allowing the membrane to detach from the glass plate. The membrane that has been detached is left to dry in an open area until it is completely dry for thickness testing and biodegradation testing.

## 2.5. Antibacterial test

The method used in testing antibacterial activity is the disk diffusion method, because this method is very simple due to its ease of use. Into the petri dish, a 1 ml bacterial suspension and liquid media are added and allowed to solidify at room temperature. After solidifying, the paper disc that has been injected with the sample solution is placed on the surface of the medium. Incubate at 37 degrees for 16-18 hours. The presence of a clear area around the medium indicates antibacterial activity. The concentrations used in the antibacterial activity test of *Escherichia coli* were 50%, 45%, and 30%, each tested in triplicate for each concentration variation to minimize errors and obtain accurate data. The media used is NA (Nutrient Agar) media which most frequently used in testing and serves as a growth medium for bacteria. The bacteria used is *Escherichia coli*, which is a gram-positive test bacterium.

## 2.6. Membrane characterization

### 2.6.1. Thickness test

The thickness of the membrane can be measured using a micrometer (precision 0.01 mm) by placing the membrane between the jaws of the micrometer. For each membrane sample to be tested at five different points, the average is then calculated.

### 2.6.2. Biodegradation test

Biodegradation analysis of cellulose acetate membrane sheets with the addition of palm oil mistletoe leaf extract was conducted by burying the cellulose acetate membrane sheets with the addition of palm oil mistletoe leaf extract in the soil with a size of 5x5 cm at a soil depth of 15 cm. The measurement process was carried out over 7 days. Before being buried, the membrane was weighed. Then buried in the ground for 7 days (Suryadinata & Putra, 2023).

## 3. Result and Discussion

### 3.1. Results

#### 3.1.1. Sample preparation

Table 2. Sample preparation

| Sample           | Extract mass (g) | Yield (%) |
|------------------|------------------|-----------|
| Fresh mass       | 1000             | -         |
| Dried mass       | 250              | 25%       |
| Extraction yield | 53.59            | 21.43%    |

The raw mass of oil palm leaf mistletoe in fresh condition is about 1 kg and dried for obtaining 250 g the dried sample as shown in Table 2. The final yield of *Ficus heteropluera* Blume extract is about 21.43 of fresh leaf.

#### 3.1.2. Phytochemical test

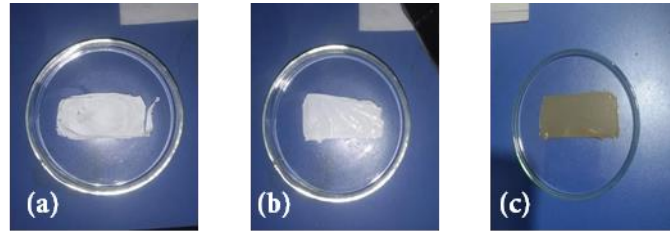
Table 3 present phytochemical test results. It reveal that oil palm leaf mistletoe has been proven to contain saponin, tannin, phenolic, flavonoid, and seroid/triterpenoid compounds. While the alkaloid compound is absent in the sample.

Table 3. Results of phytochemical testing of ethanol extract from oil palm leaf mistletoe (*Ficus heteropluera* Blume)

| Compound               | Reagent   | Phytochemical Test |
|------------------------|---|--------------------|
| Alkaloid               | HCl and Mayer   | -                  |
| Saponins               | Ethanol 70% and HCl                                   | +                  |
| Tannin                 | Aquades and FeCl <sub>3</sub> 1%                      | +                  |
| Phenolic               | Ethanol 70% and FeCl <sub>3</sub> 5%                  | +                  |
| Flavonoid              | Ethanol, HCl and Mg powder                            | +                  |
| Steroids/Triterpenoids | Ethanol 70%, Ether and H <sub>2</sub> SO <sub>4</sub> | -                  |

#### 3.1.3. Membrane thickness

The visual display of the membrane is shown in Figure 1. The higher extract *Ficus heteropluera* Blume is darker than the other samples. While, the thickness of the CA:Extract-1 and CA:Extract-2 is bigger than the CA sample.



**Figure 1.** The fabricated membrane. (a) CA with thickness of 0.03 mm; (b) CA:Extract-1 with thickness of 0.06 mm, and (c) CA:Extract-2 with thickness of 0.09 mm.

#### 3.1.4. Antibacterial test

The results of the antibacterial test against *Escherichia coli* are shown in Table 4 and Table 5. The membrane is fabricated with the addition of an additive, namely palm oil mistletoe leaf extract at concentrations of 50, 45, and 30%.

**Table 4.** Antibacterial test results of membrane CA:Extract-1 against *Escherichia coli*

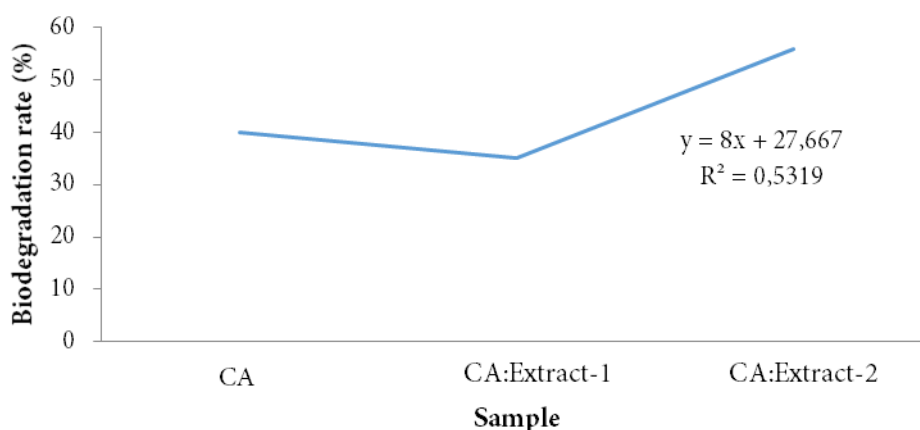
| Concentration (%) | Inhibition zone diameter (mm) |      |      | Average diameter of inhibition zone | Interpretation category activity |
|-------------------|-------------------------------|------|------|-------------------------------------|----------------------------------|
|                   | I                             | II   | III  |                                     |                                  |
| Control (-)       | -                             | -    | -    | -                                   | -                                |
| Control (+)       | 36.6                          | 38.3 | 40.1 | 38.3                                | Very strong                      |
| 50%               | 6.6                           | 7.6  | 0    | 4.73                                | Weak                             |
| 45%               | 5.2                           | 7.7  | 0    | 4.3                                 | Weak                             |
| 30%               | 6.9                           | 6.1  | 0    | 4.33                                | Weak                             |

**Table 5.** Antibacterial test of membranes CA:Extract-2 against *Escherichia coli*

| Concentration (%) | Inhibition zone diameter (mm) |      |      | Average diameter of inhibition zone | Interpretation category activity |
|-------------------|-------------------------------|------|------|-------------------------------------|----------------------------------|
|                   | I                             | II   | III  |                                     |                                  |
| Control (-)       | -                             | -    | -    | -                                   | -                                |
| Control (+)       | 39.1                          | 38.1 | 37.9 | 38.4                                | Very strong                      |
| 50%               | 0                             | 0    | 0    | 0                                   | No                               |
| 45%               | 0                             | 0    | 0    | 0                                   | Very weak                        |
| 30%               | 0                             | 0    | 0    | 0                                   | Very weak                        |

#### 3.1.5. Biodegradation test

The result of biodegradability test of the membrane is presented in Figure 2. All the sample is shown significant degradation more than 30% after a week treatments.



**Figure 2.** Graph of biodegradable membrane test results.

### 3.2. Discussion

The extraction of palm mistletoe leaves is carried out using the maceration method. The preparation of the *simplicia* from the leaves of the oil palm mistletoe (*Ficus heteropleura* Blume), which were obtained from Rokan Hulu, Riau. The powdered leaves of the oil palm mistletoe, weighing 250 grams, were macerated with 1000 ml of 96% ethanol in a dark bottle for 3x24 hours, occasionally stirred, and then filtered. From the results of the maceration, a total of 4000 ml of macerate was obtained. The solvent evaporation was carried out using a rotary evaporator under vacuum at a temperature of 78 °C. The thick extract obtained was 53.59 grams with a yield of 21.436%. The obtained extract is dark green in color. According to Hanni (2016), the advantages of the maceration method were chosen because this method is easy, does not require special skills, and can be used for *simplicia* in fine or coarse form without needing to use this method. The solvent used in this study is 96% ethanol. Phytochemical tests to provide an overview of the groups of metabolites contained in the 96% ethanol extract of oil palm mistletoe leaves. The results of the phytochemical tests shown in Table 3.2 indicate that the 96% ethanol extract of oil palm mistletoe leaves contains compounds from the flavonoid, saponin, tannin, and phenolic groups. In the phytochemical screening test, the flavonoid test yielded a positive result indicated by the appearance of a dark red color and the presence of a red precipitate, while the saponin test yielded a positive result indicated by the formation of constant foam. In the tannin test, a positive result was indicated by the formation of a brownish-green color, while the phenolic test showed a positive result marked by a green color despite some brownish hues. In the steroid/triterpenoid test and the alkaloid test, negative results were obtained because no color change occurred in the 90% ethanol extract test of oil palm mistletoe leaves.

The resulting membrane is in the form of variations of fine sheets, white in color, and stored in a membrane storage container soaked in ethanol for 10 minutes. The cellulose acetate membrane with the addition of palm oil mistletoe leaf extract produced is shown in Figure 2 qualitatively, with the DMAc solvent, the cellulose acetate membrane has a less rough surface than the membrane without the extract. Additionally, the color of the membrane with the addition of extract from the palm oil mistletoe leaf tends to be browner in the membrane with the addition of extract from variation CA:Extract-1 compared to the addition of extract from variation CA:Extract-2. The measurement of thickness primarily aims to determine the extent to which the membrane shrinks after drying. The concentration of the polymer, where an increase in polymer concentration also leads to an increase in membrane thickness, is a factor that affects the membrane.

The addition of palm oil mistletoe leaf extract received the lowest value, while the highest value was found in the membrane. The addition of extract to the cellulose in the second variation was also too much, and the pouring of the membrane onto the glass plate was uneven, resulting in an imperfect membrane printing process, which could affect the membrane thickness. The further away from the mold area, the thinner the resulting membrane. Additionally, the varying time in membrane production will affect the membrane thickness.

In this antibacterial activity test, the sample used is a membrane with the addition of an additive, namely palm oil mistletoe leaf extract. Where the extract of oil palm mistletoe leaves is very suitable for membrane production because the extract of oil palm mistletoe leaves contains phenolic compounds, saponins, tannins, and flavonoids. The concentrations used in the antibacterial activity test against *Escherichia coli* were 50%, 45%, and 30%, each tested with three repetitions for each concentration variation to minimize errors and obtain accurate data. The media used is NA media (Nutrient Agar), which is most commonly used in testing and serves as a growth medium for bacteria. The bacteria used are *Escherichia coli*, which is a Gram-negative test bacterium. The method used in the antibacterial activity test is the disk diffusion method, as this method is very simple due to its ease of use. The measurement results with the inhibition zone against the growth of *Escherichia coli* bacteria in variation CA:Extract-1 have a nearly uniform diameter in all test repetitions. In variation CA:Extract-1, the average diameter at a concentration of 50% was 4.73 mm, at a concentration of 45% it was 4.3 mm, and at a concentration of 30% there was a bacterial inhibition zone of 4.33 mm. The positive control concentration of chloramphenicol has an average diameter of 38.3 mm, and the negative control DMSO does not have a clear zone. In variation CA:Extract-2, the measurement results using a caliper did not show any inhibition zone around the disc paper containing variation CA:Extract-2. Whereas in the positive control, chloramphenicol shows.

## Conclusion

The phytochemical screening test of the oil palm mistletoe leaves revealed that the flavonoid test, saponin test, tannin test, and phenolic test yielded positive results. Meanwhile, the steroid/triterpenoid test and the alkaloid test yielded negative results because there was no color change or precipitate in the 90% ethanol extract test. The measurement results with the inhibition zone against the growth of *Escherichia coli* bacteria in variation CA:Extract-1 have an average diameter at a concentration of 50% of 4.73 mm, at a concentration of 45% of 4.3 mm, and at a concentration of 30% there is a bacterial inhibition zone of 4.33 mm. The positive control concentration of chloramphenicol has an average diameter of 38.3 mm. In variation CA:Extract-2, the measurement results did not show any inhibition zone around the disc paper. The measurement results for the positive control, chloramphenicol, showed an average diameter of 38.367 mm. The highest biodegradation test was found in the variation with the addition of 0.08 grams of extract, which was 56%, and the lowest biodegradation value was found in the variation with the addition of 0.04 grams of extract, which was 35%.

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